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Increased Plasma-Soluble Fibrin Monomer Levels in Patients With Disseminated Intravascular Coagulation

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Plasma-soluble fibrin monomer (SFM) level in patients with disseminated intravascular coagulation (DIC) was significantly higher than the level in patients with pre-DIC or in non-DIC patients, and the level in patients with pre-DIC was significantly higher than that in non-DIC patients. There was no significant difference in plasma SFM levels among various diseases underlying DIC. Plasma SFM level in patients with good outcome was significantly decreased after treatment for DIC. The sensitivity of fibrin degradation products and platelet number was high for DIC, but not for pre-DIC. The sensitivity of thrombin–antithrombin III complex, plasmin–plasmin inhibitor complex, and SFM was high for both DIC and pre-DIC. The specificity of these markers was also high. Receiver operating characteristic analysis suggests that plasma SFM level could be the most useful marker for the diagnosis of both DIC and pre-DIC. © 1996 Wiley-Liss, Inc.

Key words: DIC, pre-DIC, SFM, TAT, PIC

INTRODUCTION

Disseminated intravascular coagulation (DIC) [1,2] is associated with severe organ failure and bleeding tendency, the extent of which is thought to be related to the outcome of DIC. Therefore, early diagnosis and early therapy are required to improve treatment of this disorder. Thrombin–antithrombin III (AT III) complex (TAT) [3], plasmin–plasmin inhibitor complex (PPIC) [4], and FDP-D-dimer [5], which are sensitive indicators of coagulation activation or secondary fibrinolysis, have recently been shown to be helpful in the diagnosis of DIC and thrombotic diseases. Elevated levels of circulating soluble fibrin monomer (SFM) in plasma indicate that thrombin has converted fibrinogen to fibrin. To detect thromboembolic events in patients, various methods for measuring fibrin have been proposed [6–9]. These methods are not yet satisfactory for clinical use. Recently, a sandwich enzyme immunoassay for SFM in plasma has been developed [10]. In this study, we used this method to examine plasma SFM levels in patients with DIC, those with pre-DIC, and those without DIC.

MATERIALS AND METHODS

We examined hemostatic abnormalities retrospectively in 149 patients suspected of having associated DIC throughout their clinical course. Consecutive patients being evaluated for DIC in our hospitals over the 3-year period were examined. Of these patients, 74 were diagnosed as having DIC, in accordance with a modified version of the criteria established by the Japanese Ministry of Health and Welfare [11,12] (Table I), while 75 patients were not so diagnosed (non-DIC). Of the 74 DIC patients, 46 satisfied the DIC criteria at first. Although the other 28 patients did not satisfy these criteria initially, their DIC score (Table I) increased during their clinical course and they were subsequently

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TABLE I. Diagnostic Criteria for Disseminated, Intravascular Coagulation*

		Score (points)
1. PT ratio	1.25 – 1.66	1
	1.67 <	2
2. Fibrinogen	100 – 150	1
(mg/dl)	100 >	2
3. FDP	10 – 20	1
(μg/ml)	20 – 40	2
	40 <	3
4. Platelet count	8 – 12	1
($\times 10^4$/μl)	5 – 8	2
	5 >	3
5. Bleeding symptoms	(+)	1
6. Organ failure due to thrombosis	(+)	1

*In leukemic patients, the sum of the scores for 1, 2, 3, and 6 was 4 or higher. In nonleukemic patients, the sum of the scores for 1, 2, 3, 4, 5, and 6 was 7 or higher. The ISI of the PT reagents was 2.25.

TABLE II. Subjects

Disease	DIC	Pre-DIC	Non-DIC
Leukemia	31	14	24
AML	7	4	9
APL	9	1	2
AMMoL, AMoL	3	2	4
CML, bc	2	1	2
ALL	6	4	4
NHL stage IV	4	2	3
Solid cancer			
Stomach	7	2	9
Lung	5	2	4
Hepatoma	3	1	5
Esophagus	2	0	2
Colon	3	1	3
Breast	2	0	3
Sepsis	21	8	20
Total:	74	28	75

AML, acute myeloblastic leukemia; APL, acute promyelocytic leukemia; AMMoL, acute myelomonocytic leukemia; AMoL, acute monocytic leukemia; CML, bc, chronic myelocytic leukemia, blastic crisis; ALL, acute lymphocytic leukemia; NHL, non-Hodgkin's lymphoma.

diagnosed as having DIC. In these 28 patients, we defined pre-DIC as the condition at least 1 week before the onset of DIC [12] (Table II). Plasma SFM in 20 healthy volunteers was measured as a control. Organ failure was considered to have occurred in the lung where three conditions were present: $PaO_2 \leq 50$ mm Hg; in the kidney, creatinine ≥ 3 mg/dl, symptoms of shock on heart failure were present, and when the patient was in a coma or responded only to pain. All patients were treated with 2,000 mg/day of gabexate mesilate (FOY) for more than 1 week [13,14]. Hemostatic examinations were carried out before treatment and at 1, 3, 5, 7, 10, and 14 days after completion of treatment. The efficacy of the DIC treatment was assessed after 7 days' treatment, using the DIC scores shown in Table I, as follows: I (complete remission), DIC score was reduced to less than that conforming with DIC criteria; II (partial remission), DIC score was decreased and symptoms were improved, but findings still conformed to the DIC criteria; III, neither symptoms nor DIC score were changed; IV (exacerbation), DIC score was increased or symptoms became worse; V (death), the patient died due to DIC within 7 days of initiation of treatment; I and II were defined as good outcome; and IV and V were defined as poor outcome.

The methods used for measuring activated partial thromboplastin time (APTT), prothrombin time (PT; international sensitivity index = 2.25), and fibrin and fibrinogen degradation products (FDP) were as described previously [15]. Plasma levels of TAT, PPIC, FDP-D-dimer, and prothrombin fragment 1 + 2 (F1 + 2) were determined with Enzygnost-TAT (Behringwerke AG), PIC-test (Teijin), Frelisa D-dimer (Agen), and Enzygnost-F1 + 2 (Behringwerke AG), respectively. Plasma SFM was determined by a newly developed enzyme-linked immunosorbent assay (ELISA) method [10], briefly described below. The method is based on the two-step sandwich assay principle, using streptavidin-coated tubes as the solid phase. The same fibrin-specific monoclonal antibody, 2B5, is used both as the biotinylated capture antibody and as the peroxidase-labeled antibody. The assay is developed for the Enzymun-Test system ES300. The assay is performed at 25°C, as recommended for the ES300; 5 μl of plasma is incubated with 25 μl of 0.11 M sodium citrate for 30 min in streptavidin-coated polystyrene tubes, to which 100 μl of incubation solution (5.33 M KSCN; 0.025 M sodium phosphate, pH 7.3) is added and incubated for 30 min. One ml of biotinylated antibody, 2B5, is then added (1.3 μg/ml biotinylated antibody, 0.1 M potassium phosphate, 5.0 mg/ml bovine serum albumin (BSA), 0.5 mg/ml Tween 20, pH 7.0) and incubated for 30 min, followed by aspiration and washing with 4.3 mM NaCl solution. This is followed by the addition of 1 ml of peroxidase-labeled antibody 2B5 solution (0.14 U/ml peroxidase conjugate, 0.035 M sodium phosphate, 0.154 M NaCl, 10 mg/ml polyethyleneglycol 40,000, 2 mg/ml BSA, 0.5 mg/ml Tween 20, pH 7.4) and incubation for 30 min, after which aspiration and washing with 4.3 mM NaCl solution are performed. Then, 0.1 ml ABTS substrate solution (0.95 mg/ml 2,2′-Azino-bis(3-ethlbenzothiazoline-6-sulfonic acid), 0.06 M citric acid, 3.3 mM sodium perborate, pH 4.5) is added and incubated for 30 min. Absorbance is read at 422 nm, and the fibrin concentration is calculated from the standard curve. The monoclonal antibody (2B5) recognized the synthetic N-terminal heptapeptide (Gly-Pro-Arg-Val-Val-Val-Glu-Arg) of the fibrin α-chain. The sensitivity and specificity of the various markers were calculated as follows:

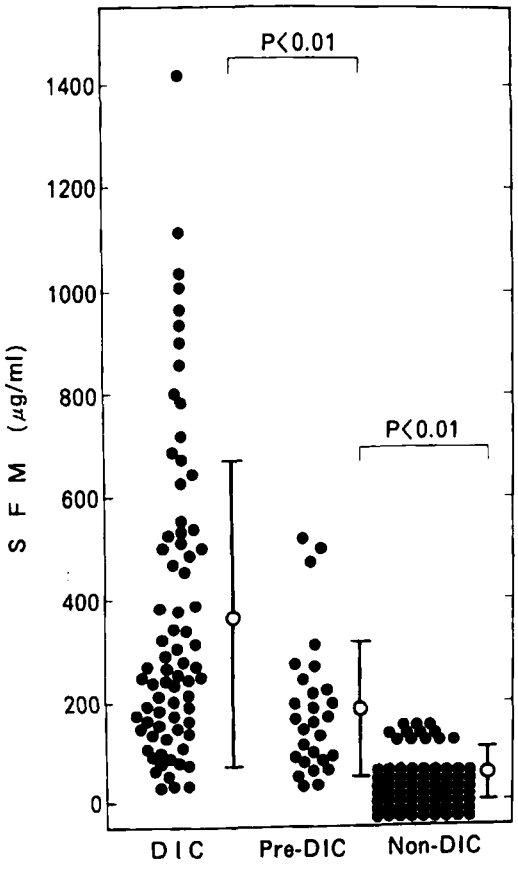

Fig. 1. Plasma SFM levels in patients with DIC, pre-DIC, and non-DIC.

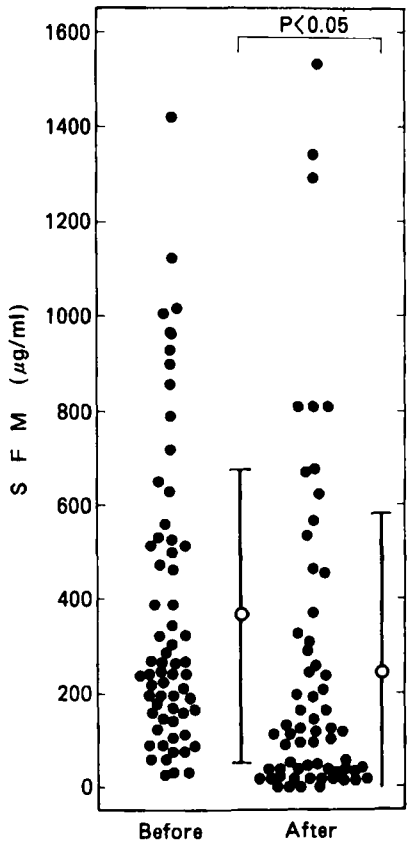

Fig. 2. Plasma SFM levels in patients with DIC before and after treatment.

Sensitivity for DIC (or pre-DIC) = number of positive DIC (or pre-DIC) patients/total number of DIC (or Pre-DIC) patients × 100.

Specificity for DIC = number of negative non-DIC patients/total number of non-DIC patients × 100.

The cutoff values were determined from the highest sum of sensitivity and specificity. The sensitivity and specificity of SFM, TAT, PPIC, FDP-D-dimer, and F1 + 2 for DIC were also evaluated by receiver operating characteristic (ROC) analysis [16]. Values are expressed as means ±SD. Statistical analysis was performed with the Wilcoxon test and Student's *t*-test. *P*-values of <0.05 in both tests were considered significant.

RESULTS

Plasma SFM level in healthy volunteers was 5.9 ± 1.4 μg/ml. The level in patients with DIC (363 ± 314 μg/ml) was significantly higher than in the patients with pre-DIC (181 ± 132 μg/ml, $P < 0.01$). Plasma SFM level was in those with pre-DIC was significantly higher than that in the non-DIC patients (52.5 ± 50.4 μg/ml, $P < 0.01$) (Fig. 1). There was no significant difference in plasma SFM levels among various diseases underlying DIC. After treatment with FOY, plasma SFM level (244 ± 340 μg/ml) was significantly decreased in DIC patients (Fig. 2). There was no significant difference in plasma SFM level between patients with good outcome (357 ± 334 μg/ml) and those with poor outcome (372 ± 282 μg/ml) before treatment. After treatment with FOY, however, plasma SFM level in those with good outcome (64.3 ± 68.4 μg/ml) was significantly reduced ($P < 0.01$), while that in patients with poor outcome (500 ± 402 μg/ml) was not reduced after treatment (Fig. 3). With regard to the sensitivity of the various hemostatic markers for DIC and pre-DIC, FDP, platelet number, TAT, PPIC, FDP-D-dimer, SFM, and F1 + 2 were positive in more than 70% of DIC patients, and TAT, PPIC, and SFM were positive in more than 60% of pre-DIC patients. In contrast, PT ratio, fibrinogen, FDP, and platelet number were negative in more than 60% of pre-DIC patients. In addition, with referrence to the specificity of the various hemostatic markers, PT ratio, fibrinogen, TAT, SFM, and F1 + 2 were negative in more than 70% of non-DIC patients. In ROC analysis for DIC patients, the sensitivity of SFM and F1 + 2 was markedly high at a low percentage of (100 − specificity). That of TAT or PPIC was low

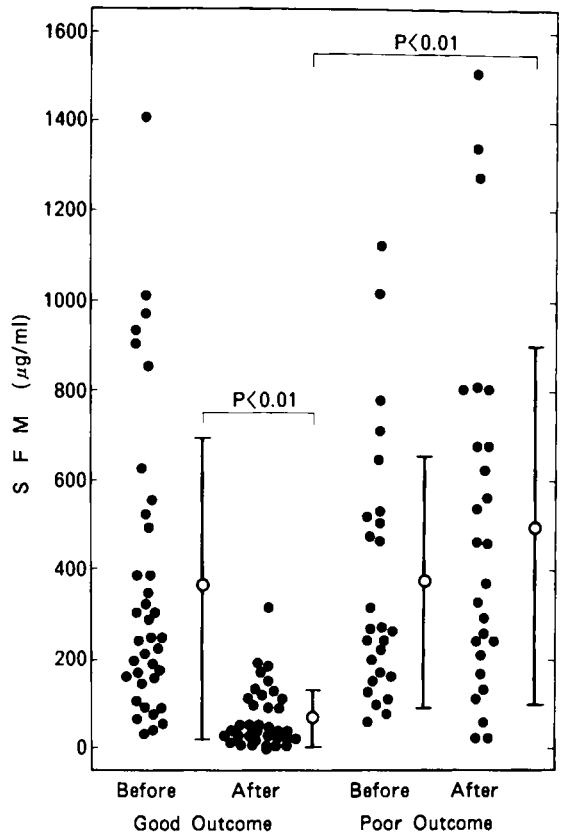

Fig. 3. Plasma SFM levels in DIC patients with good and poor outcomes.

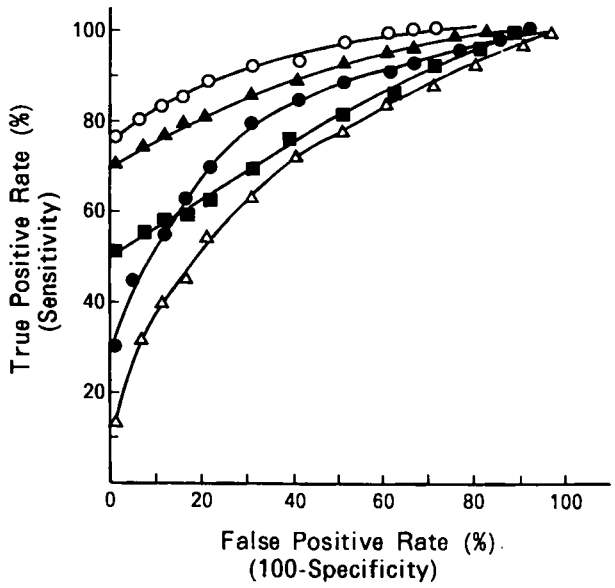

Fig. 4. ROC analysis of various hemostatic markers for DIC. Open circle, SFM; closed triangle, F1 + 2; closed circle, TAT; closed square, D-dimer; open triangle, PPIC.

at a low percent of (100 − specificity) and that became high at a high percent of (100 − specificity) (Fig. 4). In ROC analysis for pre-DIC patients, the curve of SFM was highest, and the curves of F1 + 2, TAT, and PPIC were similar, while the curve for FDP-D-dimer was lowest (Fig. 5).

DISCUSSION

Increased plasma soluble fibrin level is considered to be a molecular marker of an impending thrombotic event. Several methods of assessing soluble fibrin have been in existence for many years [6–8]. Most of these methods, however, are nonspecific, semiquantitative, or too laborious to be used in clinical practice [17]. The monoclonal antibody (2B5) in this ELISA system recognizes the epitope of the fibrin α-chain, the structure of which is exposed after removal of fibrinopeptide A from fibrinogen by the action of thrombin [10]. This immunoassay has been reported to allow specific and sensitive detection of SFM in a porcine DIC model [18].

In this study, various hemostatic parameters were observed to be altered in DIC and pre-DIC patients relative to non-DIC subjects. The routine markers (PT ratio, fibrinogen, FDP, and platelet count), as employed by the Japanese Ministry of Health and Welfare, are considered to be important markers for the diagnosis of DIC. However, these markers are not sensitive for pre-DIC. In this study, plasma SFM level was significantly different among DIC, pre-DIC, and non-DIC patients, suggesting that SFM could be an important marker for the diagnosis of both DIC and pre-DIC. The sensitivities of TAT, PPIC, FDP-D-dimer, and F1 + 2 were also high for both DIC and pre-DIC. These molecules are considered to be sensitive markers for the diagnosis of thrombosis and DIC [12,19,20]. In the outcome of DIC, greater efficacy of treatment was achieved in Pre-DIC than in DIC patients, suggesting that early diagnosis and early treatment are important [21]. In addition, these markers are sensitive and useful indicators in the early detection of DIC, i.e., for the pre-DIC stage, since TAT and PPIC levels directly reflect thrombin or plasmin generation, and FDP-D-dimer is a cross-linked fibrin derivative that mainly reflects secondary fibrinolysis. However, ROC analysis showed that the sensitivity of SFM for DIC was markedly high at high sensitivity, but that the sensitivity of TAT, PPIC, and FDP-D-dimer was high at low sensitivity. ROC analysis also suggested that SFM could be the most sensitive marker for pre-DIC. There was no significant difference in plasma SFM levels among various diseases underlying DIC, suggesting that plasma SFM level is solely dependent on fibrin formation, and not on liver function, inflammation, or bone marrow suppression. After treatment, plasma SFM level was significantly reduced in patients with good outcome but not in those with poor outcome,

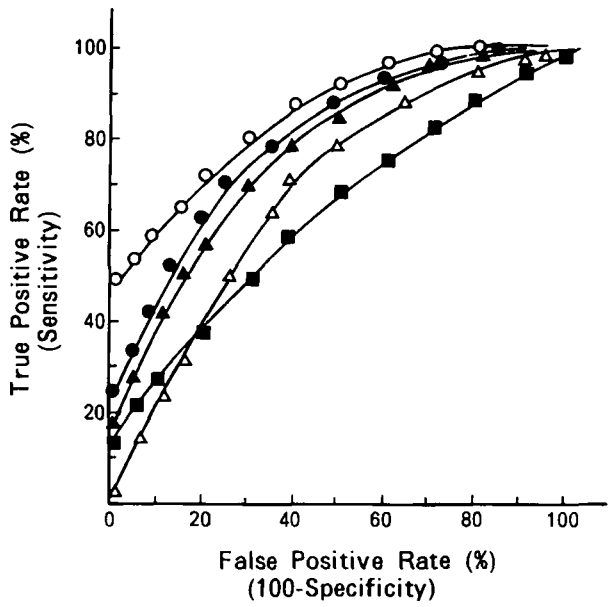

Fig. 5. ROC analysis of various hemostatic markers for pre-DIC. Open circle, SFM; closed triangle, F1 + 2; closed circle, TAT; closed square, D-dimer; open triangle, PPIC.

indicating that SFM could be a useful marker for monitoring the anticoagulant treatment of DIC. Most of the above molecular markers reflect intravascular thrombin generation or plasmin generation, and they do not directly reflect microthrombus formation, whereas plasma SFM level is a true reflection of the amount of fibrin converted from fibrinogen by thrombin. It has been shown that the half-life of SFM in plasma is longer than that of TAT or F1 + 2 [17]. We, therefore, conclude that plasma SFM could be the most useful clinical marker for the diagnosis of DIC and pre-DIC.

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